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Supplementation of *Moringa oleifera* leaf meal in feed on sexual behavior and semen quality of Landrace-cross boars

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Abstract

This study aims to determine the effect of *Moringa* leaf flour supplementation in commercial feed on sexual behavior and semen quality of cross-bred Landrace boars. This research was carried out at the UPT Baturiti Regional Artificial Insemination Center (BIBD) located in Banjar Pekarangan, Baturiti Village, Baturiti District, Tabanan Regency, Bali Province, Indonesia. Observations used a completely randomized design consisting of two treatments, with 10 repetitions (number of semen collection). The treatments tried were boar fed without the addition of *Moringa* leaf meal (P0) and boar fed with the addition of 1% *Moringa* leaf meal (P1). The results showed that the addition of 1% *Moringa* leaf flour in feed (P1) showed significantly different results ($P<0.05$) on sexual behavior. The addition of *Moringa* leaf flour in commercial feed as much as 1% obtained significantly different results ($P<0.05$) in the semen evaluation value. It was concluded that the addition of 1% *Moringa oleifera* leaf meal in commercial feed had an effect on sexual behavior and could improve the semen quality of Landrace-crossbred boars.

Keywords: Boar landrace-crossbreed; *Moringa oleifera*; Sexual behavior; Semen

1. Introduction

Pigs are one of the meat-producing livestock commodities that have great potential for development, because of their fast growth, good feed conversion and ability to adapt to various environmental conditions, and their carcass percentage can reach 65% to 80% [1].

Nowadays, efforts to increase livestock production can be made by optimizing reproductive efficiency, one of which is by carrying out artificial mating or artificial insemination (AI). Achieving the goals of an artificial insemination (AI) program depends on several factors, including the quality of the semen used [2]. Factors that influence fresh semen are sexual behavior and semen evaluation scores. Sexual behavior, including length of libido (male arousal) and time of ejaculation. The duration of libido is characterized by the male's reaction when riding the sow dummy, while the duration of ejaculation is closely related to the quantity of semen volume released.

Fresh semen does not last long in *in vitro* storage due to rapid sperm death. One of the causes of sperm death is free radical attack as a result of the electron transport process in mitochondria to the sperm plasma membrane [3]. Sexual behavior and semen evaluation scores are influenced by several factors, namely genetics, feed, temperature, age and ejaculation frequency [4]. Feed with insufficient quantity and quality can affect growth and can reduce the quality of livestock sexual behavior and the semen produced. To maintain and improve the sexual behavior and semen evaluation value of boar, feed additives are needed that are easy to obtain and contain sufficient vitamin E. One source of vitamin

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E is found in the herbal plant *Moringa oleifera* leaves. Vitamin C functions to protect sperm from oxidative stress [5] and vitamin E functions to improve the health of reproductive organs, prevent sperm cell membranes from fat peroxidation, and defend sperm from oxidative reactions [6]. Vitamin E deficiency can cause degeneration of reproductive organs and decreased sexual activity [7]. *Moringa* leaves contain vitamin A, vitamin C, alpha-carotene, arginine, beta-carotene, betasitosterol, caffeoylquinic acid, campesterol, carotenoids, chlorophyll, chromium, delta-5-avenasterol, delta-7-avenasterol, glutathione, histidine, acetic acid indole, indoleacetonitrile, kaempferal, leucine, lutein, methionine, myristic acid, palmitic acid, prolamin, proline, quercetin, routine, selenium, threonine, tryptophan, xanthine, xanthophyll, zeatin, zeasantin, zinc [8].

Optimal sexual behavior and semen evaluation values can be obtained with boar preparation, adequate feed, and boar health. Libido or the desire to mate is manifested in the form of sexual behavior, which occurs as a response from male livestock due to a stimulus. Several studies have been conducted on the use of *Moringa* leaves as animal feed and its effect on sexual behavior and semen evaluation values. Syarifuddin et al.[9] showed that giving dried *Moringa* leaves as much as 15% of the concentrate to cows could increase testosterone hormone concentration, libido and spermatozoa motility. Abu et al.[10] showed that *Moringa* leaf flour up to 15% did not have a negative effect on the quality of spermatozoa in the epididymis of rabbits. Raji and Njidda [11] stated that supplementation with 50% *Moringa* leaf feed in goats can increase sperm count. *Moringa* extract supplementation has a positive impact on boar health reproductive parameters and positively effects sperm characteristics[12].

The use of *Moringa* leaf flour to improve sexual behavior and semen evaluation value in boars has not been widely used. Therefore, research on the supplementation of *Moringa* leaf flour in feed on sexual behavior and semen evaluation in Landrace-crossed boars needs to be carried out.

2. Material and methods

2.1. Animal treatments and experimental design

The livestock used in this research were boar Landrace crosses, 4 years old in healthy condition and had good semen quality, namely a spermatozoa concentration of more than $1.50x10^6$ cells/ml and spermatozoa motility of more than 60%. Semen collection was carried out every three days. The commercial feed used in this research is the Master 1031 brand commercial feed produced by PT. Malindo Feedmill, Tbk., Indonesia. The drinking water provided comes from well water which was provided *ad libitum*. Observations used a completely randomized design consisting of two treatments, with 10 repetitions (number of semen collection). The treatments tried were boar fed without the addition of *Moringa* leaf meal (P0) and boar fed with the addition of 1% *Moringa* leaf meal (P1). Observation of the quality and quantity of semen was carried out on fresh semen of boar Landrace-cross that was collected.

2.2. Equipment

The equipment used in this research included a dummy sow, boar cage, measuring cup, water bath, elemental tube, dropper pipette, thermometer, binocular microscope, glass object, preparation box, pH meter, 2% eosin dye, stopwatch and tools. Landrace-cross boars were given commercial feed with the addition of 1% *Moringa* leaf meal. This feeding treatment was carried out for one month, then sexual behavior and semen quality were observed. Semen collection was carried out every three days. Observations of sexual behavior include length of libido and time of ejaculation, while observations of semen include fresh semen, and macroscopic and microscopic evaluations are carried out. Before collecting semen, the boar and cage were first cleaned. This was done to avoid contamination which can cause a decrease in the quality of the cemen collected. Data collection was carried out by direct observation regarding the sexual behavior of Landrace-cross boars, including observations on the length of libido and the time of ejaculation. The data collected can be used to see the correlation between giving *Moringa* leaf flour and the sexual behavior of Landrace-cross boars.

2.3. Variable measurement.

Sexual behavior includes: (i) Length of libido, measured by observing the reaction time of the boar being released from the cage to the shelter (from rising to falling from the dummy sow); and (ii) Time of ejaculation, measured by observing the time it takes for semen to drop from the reservoir (dummy sow). Characteristics of fresh semen include volume (checked from boar semen collected using a periodic collection tube); color (the color of the semen was assessed by looking directly at the semen from the boar which has been collected in the holding tube, in general the color of the semen is cloudy white, milky white, cream, yellowish cream, to grayish white); consistency (the tube containing pig semen was shaken slowly and the viscosity level was seen, with three assessment criteria, namely thin, medium and thick); mass movement of spermatozoa: using a light microscope with a magnification of 10x10, with the assessment being very good (+++); good(++); enough (+); and less (-). Spermatozoa concentration (procedure for counting

spermatozoa using a hemocytometer; percentage of spermatozoa motility (percentage of motility can be assessed subjectively by comparing motile spermatozoa moving forward (progressive) with those that were not progressive (linear). The assessment given was from 0% (not motile) to 100%); and spermatozoa viability (examination was carried out under a microscope and spermatozoa were counted in a minimum of 200 cells from 10 fields of view).

2.4. Data Analysis

The sexual behavior of Landrace-cross boars was analyzed statistically using statistical correlation tests. The analysis results obtained are then used to test the research hypothesis. Evaluation of Landrace-cross boar semen was processed using the t-test [13]. The t-test (t-Test) is one of the tests used to determine whether there is a significant difference or not ($P<0.05$).

3. Results and discussion

3.1. Sexual behavior of Landrace-crossbred boars

The results of the research showed that the sexual behavior of Landrace-cross boar fed with the addition of 1% *Moringa* leaf flour showed a significant correlation. The results of observations of the sexual behavior of Landrace-cross boars are presented in Table 1.

Table 1 Sexual behavior of Landrace-cross boars fed with the addition of *Moringa* leaf meal

Sexual behavior	**Avarage value**		**Standard**
	P0	**P1**	
Length of libido (seconds)	72.20±20.51a	37.30±11.10b	180-1200*
Ejaculation times (seconds)	468.30±79.16a	642.30±25.98	

Note: (a, b) different letters on the same line show significant differences ($P<0.05$); * Seran et al.[14]

The average duration of libido in boars obtained in this study was 72.20±20.51 seconds (P0) and 37.30±11.10 seconds (P1). These results had a significant difference ($P<0.05$) and showed that there was a significant correlation between feed without the addition of *Moringa* leaf flour and feed supplemented with 1% *Moringa* leaf flour on the length of boar libido.

The average length of boar ejaculation obtained in this study was 468.30±79.16 seconds for the P0 boar group and 642.30±25.98 seconds for the P1 boar group. These results show a significant difference ($P<0.05$) and show that there is a significant correlation between P0 and P1.

3.2. Evaluation of Landrace-cross boar cement

The results of research evaluating fresh semen of Landrace-cross boars supplemented with *Moringa* leaf meal in the feed showed significantly different results ($P<0.05$) on semen volume, cement concentration, motility and viability. Meanwhile, pH, color, consistency and mass movement of cement showed results that were not significantly different ($P>0.05$). Semen examination is carried out in a laboratory with a room temperature of 20-22°C. The average results of cement characteristics are presented in Table 2.

The average volume of semen per ejaculation obtained in this study was 323.00±24.96mL without *Moringa* leaf flour and 432.00±34.57mL, and showed a significant difference ($P<0.05$).The color of spermatozoa is determined by concentration and consistency. The higher the cement concentration, the darker the cement color and the higher the consistency. Boars have semen with a watery consistency and a milky white color. This is due to the presence of riboflavin which is produced from the secretions of the vesicular glands. The spermatozoa in this study were milky white with a watery consistency and mass movement (+++), without significant differences ($P>0.05$).

The pH value of fresh spermatozoa obtained in the study was 7.47±0.57 in the boar P0 group and 7.42±0.33 in the boar P1 group and showed no significant difference ($P>0.05$). Spermatozoa concentration is a very important parameter for determining sperm quality. The spermatozoa concentration in this study was $2.42±0.99x10^6$ cells/ml in the boar P0 group and $3.20±1.14x10^6$ cells/ml in the boar P1 group and was statistically stated to be significantly different ($P<0.05$).

Table 2 Characteristics of fresh semen in Landrace-cross boars fed with the addition of *Moringa* leaf meal

Cemen characteristic	**Avarage value**		**Standard**
	P0	**P1**	
Semen volume, mL	323.0±24.96a	432.0±34.57b	150-200*
Color	Milk white	Milk white	Milk white**
Consistency	watery	watery	Watery**
Mass movement of spermatozoa	(+++)	(+++)	(+++)**
pH spermatozoa	7.47±0.57	7.42±0.33	7.30-7.80
Spermatozoa motility, %	63.50±0.02a	70.00±0.04b	50-80*
Spermatozoa viability, %	69.75±4.41a	76.89±4.46b	70-90*
Spermatozoa concentration, 10^6 sel/ml	2.42±0.99a	3.20±1.14b	200-300*

Note: (a, b) different letters on the same line show significant differences (P<0.05); *) Garner and Hafez [15]; **) Knox [16]

The spermatozoa motility calculated in this study is progressive spermatozoa motility, namely sperm that move forward. The motility of fresh spermatozoa from boar in this study was 63.50% (P0) and 70% (P1), statistically significant different (P<0.05). The results of spermatozoa viability in the boar P0 group were 69.75±4.41% and 76.89±4.46% in the boar P1 group, statistically significantly different (P<0.05).

The libido of Landrace-cross boars in the P1 group (1% *Moringa* leaf flour) was faster than that of the P0 boar group. This indicates that there is an effect of *Moringa* leaf flour supplementation in the diet on the libido of Landrace-cross boars. This effect is caused by the vitamin content in *Moringa* leaf flour which can increase boar libido. This is in line with [17] statement that vitamin A is beneficial in terms of fertility and increasing libido. The duration of libido in boars is around 30 seconds faster than the duration of libido in cows [18]. The shorter the libido reaction, the higher the boar's sexual arousal. This is in accordance with [19] statement that the best reaction time for boars is in seconds, because the shorter the reaction time indicates the suitability of the boar for the breeding program. Apart from that, there are several factors that influence libido, namely the testosterone hormone. The testosterone hormone has a very important role in the spermatogenesis process which is produced after reaching sexual maturity when the activity of the male reproductive organs begins. This hormone is needed in the process of cell division in the meiosis phase, especially during the change from spermatids to spermatozoa [20]. Senger [21] reports that without the hormone testosterone, spermatogenesis is unlikely to occur, and a lack of steroids can cause reproductive problems in bulls. Therefore, the effect of testosterone on libido will be visible if the requirements for spermatogenesis are sufficient. Supplementing *Moringa* leaf flour in feed has been proven to increase the libido of cross-bred Landrace boars.

The research results of [14] report that the length of ejaculation in boar is between 3-20 minutes. The duration of ejaculation is closely related to the volume of semen produced. The longer the ejaculation takes, the greater the volume of semen that can be accommodated. In this study, it was revealed that supplementation of *Moringa* leaf flour in the diet was proven to increase the ejaculation time of Landrace-cross boars, so that it had a positive correlation with the volume of semen stored.

Cement evaluation is an important indicator in determining the quality and quantity of cement stored. According to [15], there are several minimum standards that fresh semen must have before dilution, namely a percentage of spermatozoa motility of more than 60%, with spermatozoa viability of more than 70% and abnormalities of less than 20%. This standard indicates good quality semen to support the fertilization process.

There was an increase in semen volume in the boar P0 group, namely 323mL to 432mL (P1). The addition of *Moringa* leaf flour in feed can increase semen production, which is in accordance with the desired cement quality standards. This increase was due to the role of the starch content of *Moringa* leaves in having a positive impact on boar reproductive productivity. Research by [22] found that giving feed supplements containing certain ingredients can increase semen volume in bulls. In addition, research by [23] reported that nutritional manipulation through providing feed supplements can increase semen production in boars. The positive implications of increasing semen volume in the context of livestock reproduction cannot be ignored.

A larger semen volume can increase the chances of successful fertilization and pregnancy in female animals [24]. In addition, increasing semen production can support breeding programs, such as artificial insemination or AI to select superior genetic sources to improve the quality and productivity of pigs. Therefore, the finding that treatment with *Moringa* leaf flour increases semen volume in Landrace-cross boar is very significant and has positive implications in livestock reproduction management and breeding.

The color of the semen obtained in this study was generally milky white with a watery consistency. This is in accordance with what [25] reported that the color and consistency of pig semen depends on the fraction that is collected, namely the pre-spermatozoa fraction is watery with a white-grey color, and the spermatozoa-rich fraction is like non-thick milk (milky-nonviscous) with a creamy white color, and is in line with the opinion of [26] which states that boars have watery sperm and are milky white in color.

The consistency of the fresh cement obtained is thin, with very good mass movement (+++).The results obtained are in accordance with the opinion of [16] who stated that boar cement has a milky white color and a runny cement consistency. Semen color is closely related to the consistency and concentration of spermatozoa. Deeply colored semen indicates the consistency and high concentration of spermatozoa [27].

Changes in semen pH from 7.47 (P0) to 7.42 (P1) also reflect positive adaptation in environmental conditions that support sperm quality and survival. This result is in line with the research results of [28], namely the pH of boar cement ranges between 7.4±0.2 [15] and pH 7.2-7.5 [29]. If there is a decrease in pH, the metabolism and motility of spermatozoa will also decrease [28]. Johnson et al. [29] added that pH below 7.2 will reduce spermatozoa motility. Differences in physiological pH values can be caused by differences in race, environment, and differences in buffers. This is the basis for making diluent solutions, because the pH of the solution can affect the viability of spermatozoa. The degree of acidity (pH) can affect the viability of spermatozoa. Lower or higher than normal pH can cause spermatozoa to die more quickly. Changes in pH can occur, because cement is left exposed to room temperature without being diluted.

Increased motility and viability of spermatozoa also reflects an increase in overall sperm quality. Motility that increases from 63% (P0) to 70% (P1) indicates an increase in sperm movement ability, which is very important in the fertilization process. Likewise, the increase in spermatozoa viability from 69.75% (P0) to 76.89% (P1) indicates that sperm have a better ability to survive and function well in the reproductive process.

Previous researchers have confirmed the close relationship between spermatozoa motility and viability and sperm fertilization ability in livestock [30]. Good motility allows sperm to swim quickly and efficiently towards the egg, while high viability ensures that the sperm has enough strength and endurance to survive the long journey to the uterus and egg [31]. Tanga et al. [32] reported that increasing spermatozoa motility and viability was positively correlated with the success rate of fertilization in livestock, indicating the importance of optimal sperm quality in achieving pregnancy. The spermatozoa concentration obtained in this study was higher than the normal concentration according to [15,25], namely ranging from 200-300x10^6 cells/ml. Factors that cause differences in concentration are feed and shelter intervals [29].

4. Conclusion

It was concluded that supplementation of 1% *Moringa oleifera* leaf meal in feed can increase the sexual libido of Landrace-cross boars and can improve the quality of their semen.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The material used in this research has been approved by the Animal Ethics Commission, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia.

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